

Effect of drying process on antioxidant properties of date palm fruits

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Aim. In order to identify antioxidant activities and phenolic compound, two varieties of date palm (Mazfati and Kalute varieties) (*Phoenix dactylifera*) fruits (DPF) from Iran systematically evaluated.

Methods. Antioxidant activity determined using typical methods such as DPPH, reducing power and total antioxidant method. The total phenolic content of the dates was measured using Folin-Ciocalteu method. The included samples were gathered at three stages of khalaal, rutab, tamr and dried date from Bam and Jiroft date. The total phenolic content ranged from 1074, 856.4 and 723.8 in Mozafati variety and 921.5, 723.5 and 785.3 mg gallic acid equivalents (GAE/100-g-dw sample) in Kalute variety for khalal, rutab and tamr stage, respectively.

Results. In both varieties antioxidant activities and total phenolic content decreased by ripening stages. Result of drying process showed that total phenolic content and antioxidant activities varied from temperature and decreased by increase of drying temperature.

Conclusion. This research demonstrates Iranian dates could be potential rich resources of natural antioxidants, and could be developed into functional foods or drug for the prevention and treatment of diseases caused by oxidative stress.

KEY WORDS: Fruit - Antioxidants.

Reactive oxygen species may cause a broad spectrum of damage to biological systems, and oxidative stress plays an important role in many chronic and degenerative diseases, such as cardiovascular diseases, cancer, diabetes mellitus and ageing.¹

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Natural antioxidants may come from vegetables, fruits and beverages. As an important category of phytochemicals, phenolic compounds universally exist in plants.^{2, 3}

The antioxidants constitute a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules, such as proteins, lipids, carbohydrates, and DNA.⁴ Many of those substances are plant-derived natural molecules that contribute to the prevention and treatment of diseases in which reactive oxygen species are involved. This protection can be explained by the plant antioxidants' capacity to scavenge free radicals.^{5, 6}

Generally, dietary plants and plant products are rich sources for natural phytochemical antioxidants including vitamins (ascorbic acid, vitamin A and α -tocopherols), carotenoids and phenolic compounds.^{7, 8} Fruits and vegetables have been implicated in preventing or reducing the risk of coronary

heart diseases, cancer and other chronic diseases.⁹⁻¹¹ For these reasons, recommendations to increase the dietary intakes of fruits and vegetables have been suggested by many world authorities.¹²

The fruit of the date palm (*Phoenix dactylifera*) is an important commercial crop in the Middle Eastern countries. Dates are still considered by many people in this part of the world as a staple food.¹³ Date palm is a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium and a significant amount of calcium.^{14, 15} The nutritional and biochemical aspects of dates were reported by many workers.¹⁵⁻¹⁷ They are rich in simple sugar such as glucose and fructose (65-80%), and a good source of fibers and some essential minerals, but low in fat and protein with no starch.¹⁷ The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due to the potential health benefits of these polyphenolic compounds as important dietary constituents.¹⁸

Besides nutritional value, date fruits are rich in phenolic compounds including antioxidant and antimutagenic properties *in vitro*.¹⁹ Date palm fruit development takes place through basically 4 stages named by their Arabic denominations, kimri, khalaal, rutab and tamr. At the kimri stage there is a rapid increase in size, weight, and reducing sugars; it is the period of highest acid activity and moisture content (up to 85%). All mentioned factors level off at the end of this stage when the fruit starts to turn yellow or red according to variety. At the khalaal stage weight gain is slow but sucrose content increases, moisture content goes down, and tannins will start to precipitate and lose their astringency. When (normally) the tips of the fruit starting to turn brown, the rutab stage sets in which is characterized by a decrease in weight due to moisture loss, a partial (the degree depending on the variety) inversion of sucrose into invert sugar and a browning of the skin and softening of the tissues. The moisture content goes down to about 35% and the dates at this stage are sold as fresh fruit. Only when the dates are left to ripen further on the palm will they turn into tamr, climatic conditions permitting, characterized by a moisture content at which the date is self-preserving. The upper limit of moisture content for the date to be self-preserving lies at around 24-25%.²⁰ Recently, several studies have reported such activity of date fruits from Algeria,²¹ Kuwait,²² Oman¹⁵ and the USA.²³ These studies showed that fresh and dried dates varied quantitatively and qualitatively in

their phenolic acids content. Such variations are a reflection of the diversity of date cultivars. More information about the antioxidant activity of various date cultivars at different maturity stages and the relationship of such activity with chemical constituents is needed.

The importance of antioxidants in maintaining health and protection from coronary heart disease and cancer is of great interest among scientists, food manufacturers and consumers, which should further intensify the interest in revealing the antioxidant properties of fruits.⁴

The aim of the present study was evaluation of the potential antioxidant activity, using the DPPH scavenging method, reducing power assay and total antioxidant capacity and also estimation of the phenolic content using the Folin-Ciocalteu method at three ripening stages and dried fruit (sun dried and oven dried).

Materials and methods

Plant material

Two varieties of date were used in this study, Jiroft Kalute date and Bam Mozafati date that are grown mostly in Kerman Province of Iran. The samples were selected identically in term of ripening stages. The dates were obtained from Jiroft distribution center at different ripening stage.

Chemicals and reagents

DPPH, trichloro acetic acid (TCA), gallic acid, sodium carbonate, Folin-Ciocalteu's phenol reagent, methanol, phosphate buffer, potassium ferricyanide, sulphuric acid, sodium phosphate and ammonium molybdate were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

Drying of dates

Fresh matured date palm fruits were weighed and dried in cross flow air oven drier (Model OV-160) at different temperatures (50 °C, 60 °C, 70 °C, and 80 °C) and air velocity of 1.5 m²/s for 48 h in same orientation of product.²⁴ Drying process started when the drier reached to constant temperature. Dry weight was determined according to the AOAC (1990) method.²⁵ For preparation of sun dried sam-

ples, date fruit samples were kept in subject to sun (at average temperature 25-45 °C) on a wood plate, for 1 week.

Extraction of antioxidants from date fruit

The flesh part of the date (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dry-blended for 3 min with a domesticated blender (Panasonic, Penang, Malaysia). The extraction solvent was 300 mL methanol-water (4:1 v/v), and extraction carried out at ambient temperature (20 °C) for 24 h using a laboratory shaker. The ratio of methanol and water which resulted the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by and Biglari *et al.*^{20, 21} Each extract was filtered with Whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the extracts were dried by a freeze dryer and dried sample constituents stored at 4 °C until use.²⁶

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin-Ciocalteu micro method.²⁷ Briefly, 20 µL of extract solution were mixed with 300 µL of Na₂CO₃ solution (20%), then 1.16 mL of distilled water and 100 µL of Folin-Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation were obtained from calibration curve:

$$A = 0.98 C + 9.321 \times 0.001 \quad (1)$$

$$R^2 = 0.9965$$

Where A is the absorbance and C is concentration as gallic acid equivalents (µg/mL).

Reducing power assay

The ability of extracts to reduce iron (III) was assessed by the Yildirim *et al.* (2000) method.²⁸ The dried extract (125-1000 µg) in 1 mL of the corre-

sponding solvent was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (K₃Fe(CN)₆; 10 g /1), then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 mL of trichloroacetic acid (100 g/1) were added and the mixture was centrifuged at 1650 g for 10 min. Finally, 2.5 mL of the supernatant solution were mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (1 g/1) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Blois (1958) method.²⁹ Briefly, 1 mL of a 1 mM methanolic solution of DPPH was mixed with 3 mL of extract solution in methanol (containing 50-400 µg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (30). An aliquot of 0.1 mL of sample solution (containing 100-500 µg of dried extract in corresponding solvent) was combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. the absorbance was measured at 695 nm against a blank when the samples had cooled to room temperature. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The effect of

ripening stages and drying process on antioxidant activities of two dates varieties were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5% significant level using the SAS 9.2 Software program.

Result and discussion

Total phenolic compound (TPC)

Date ripen in four stages, which are known throughout the world by their Arabic denominations; kimri (unripe), khalal (full-size, crunchy), rutab (ripe, soft) and tamr (ripe, reduced moisture). The date goes from one extreme of moisture content of 85% at early Kimiri stage to 50-60% for Khalal, about 35-40% for Rutab, and about 20% for Tamr. Due to variety and growth conditions, DPF vary in shape, size, weight and moisture content. Some of study reported that phenolic substance (referred to generically as tannins) were high in the inedible Kimri stage of date and declined progressively as the date matured to Tamr stage.³¹⁻³³ The averages of total phenolic compound of DPF based on Folin-Ciocalteu method were shown in Figure 1.

As can be seen from Figure 1 date varieties have significant differences ($P < 0.05$) in total phenolic content. Among studied varieties, Mozafati contained the higher amount of total phenolic in comparison with Kalute. These results showed that date

palm fruit grown in kerman had a similar level of phenolic content with those of Tunisia date palm fruit³⁴ and Oman date palm fruit and also with those of Bahrain dates.³⁵ However, Mansouri *et al.* (2005) and Biglari *et al.* (2008) reported that total phenolic content of Algerian and Iranian date palm fruit ranged from 2.49-8.36 mg GAE/100 g of fresh weight and from 2.89 to 6.64 mg GAE/100 g of dry weight respectively.^{20, 21} These levels are much lower than this study, except for Kharak date (Iranian dry date) that showed an average of 141.35 mg GAE/100 g dry weight. In the other way, the study reported by Wu *et al.* (2004), on lipophilic and hydrophilic antioxidant capacities of common foods in the United States found that Deglet Noor and Medjool varieties presented a high level on total phenolic content (661 and 572 mg of GAE per 100 g fresh weight respectively) as compared to our study.³⁶ Various factors such as variety, growing condition, maturity, season, geographic origin between the two countries, fertilizers, soil type, amount of sunlight received and experimental conditions (storage, extraction) among others might be responsible for the observed differences. The order of total phenolic content of date samples is:

Sun dried date < tamr stage < rutab stage < khalal stage.

About variation of TPC in ripening stages, Myhara *et al.* (2000) reported that phenolic substance (referred to generically as tannins) were high in the inedible Kimri stage of dates and declined progressively as the dates matured to Tamr stage.¹⁷

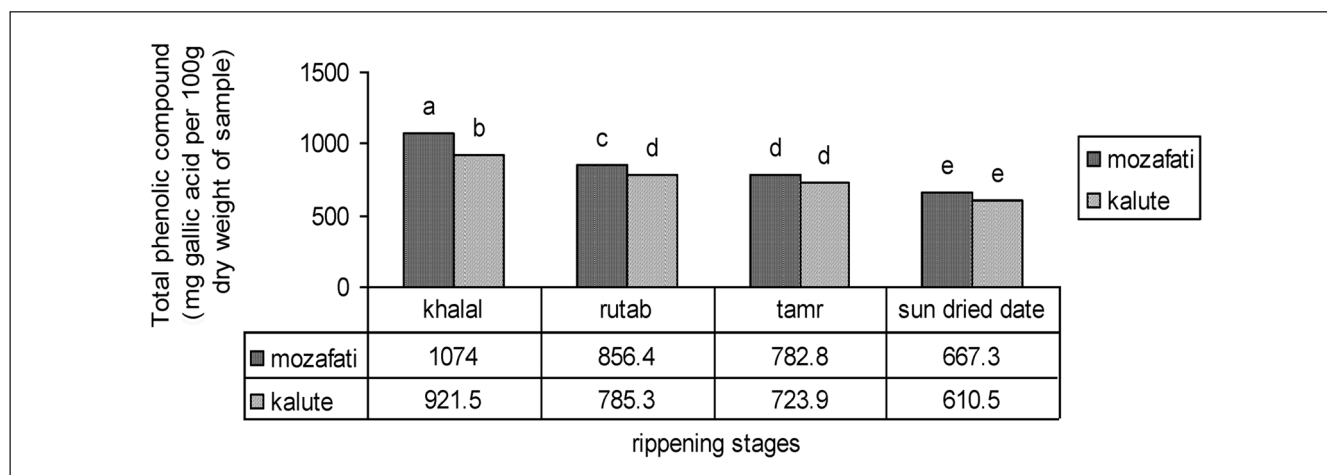


Figure 1.—Effect of ripening stage and sun drying on total phenolic compound of two DPF varieties.

As can be seen from Figure 2, date varieties and drying temperatures had significant effect on ($P < 0.05$) total phenolic content. By increasing in drying temperature, total phenolic compound decreased. No result was reported about the effect of drying temperature on TPC but Allait (2008) showed that dried date containing lower TPC than fresh date.³⁵

Antioxidant activity

TOTAL ANTIOXIDANT ACTIVITY

In the phosphor molybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the three extracts concentration exhibited

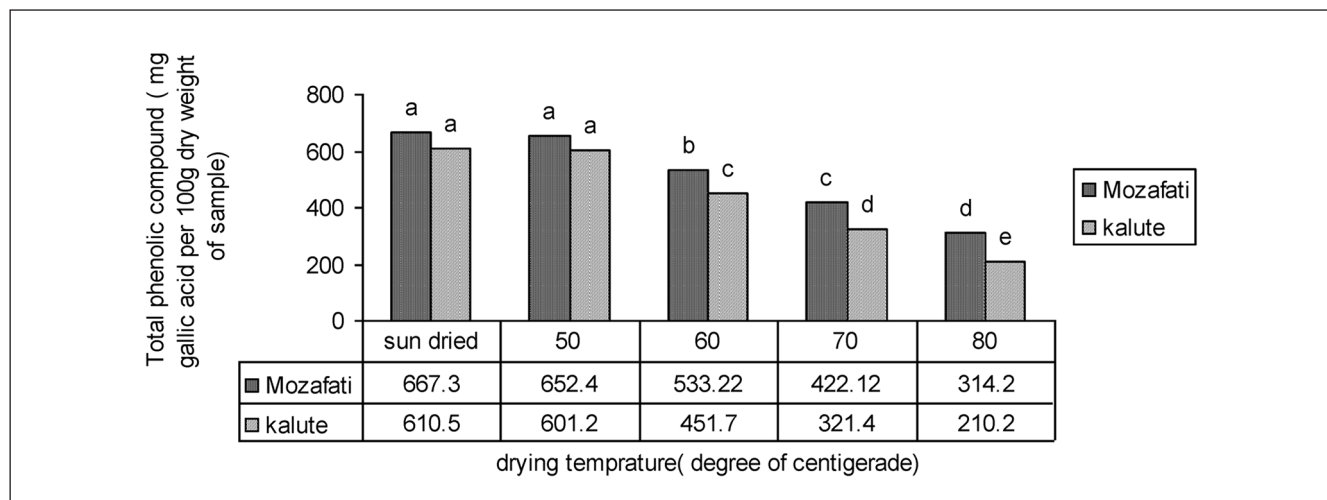


Figure 2.—Effect of drying temperature on total phenolic content of two date variety.

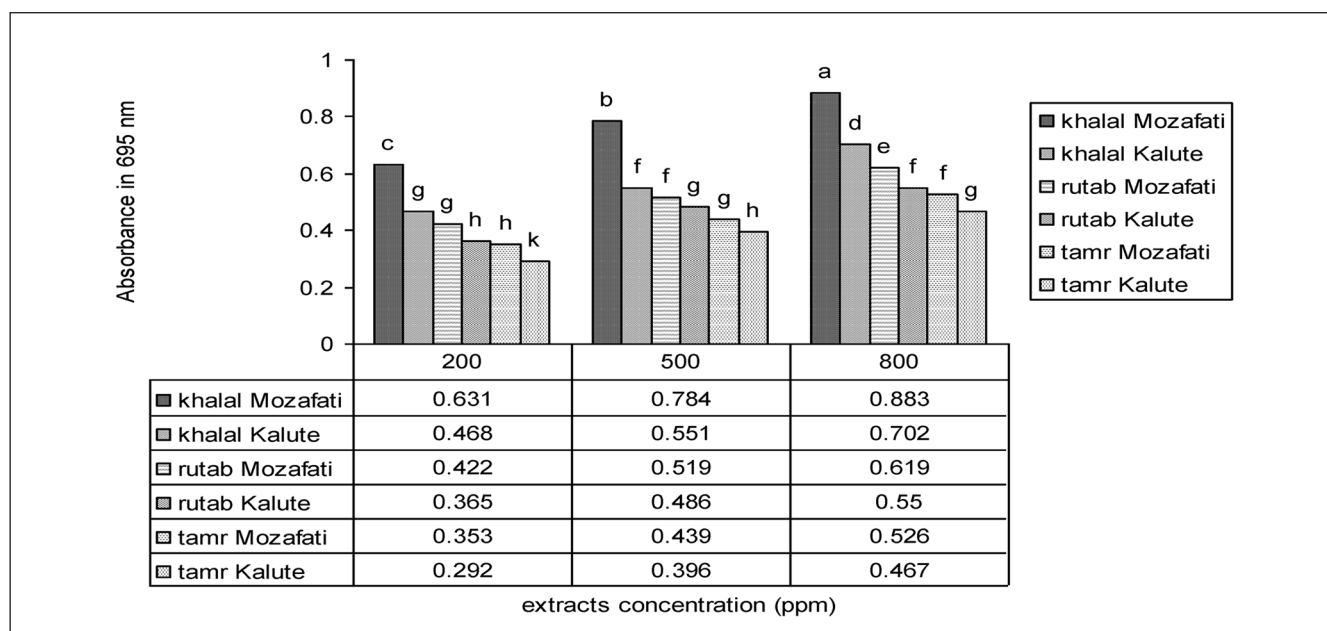


Figure 3.—Total antioxidant capacity of date varieties during ripening stages.

some degree of activity in a dose-dependent manner. The results of total antioxidant capacity of date during ripening stages were shown in Figure 3. As it can be seen from Figure 3, in this study, total antioxidant capacity decreased during ripening stages and increased with increase of extract concentration.

Figure 4 showed the effect of drying process on the total antioxidant capacity of dried date in 500 ppm concentration. Results showed that total antioxidant capacity was decreased with increase of drying temperature and no significant differences ($P < 0.05$) observed between total antioxidant capacity

of sun dried date and oven dried date (oven in 50°C).

REDUCING POWER ASSAY

The results of reducing power of extracts in ripening stages were shown in Figure 5. Different studies have indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with antioxidant activity.^{37, 38} In this assay, the ability of extracts to reduce iron (III) to iron (II) was determined.

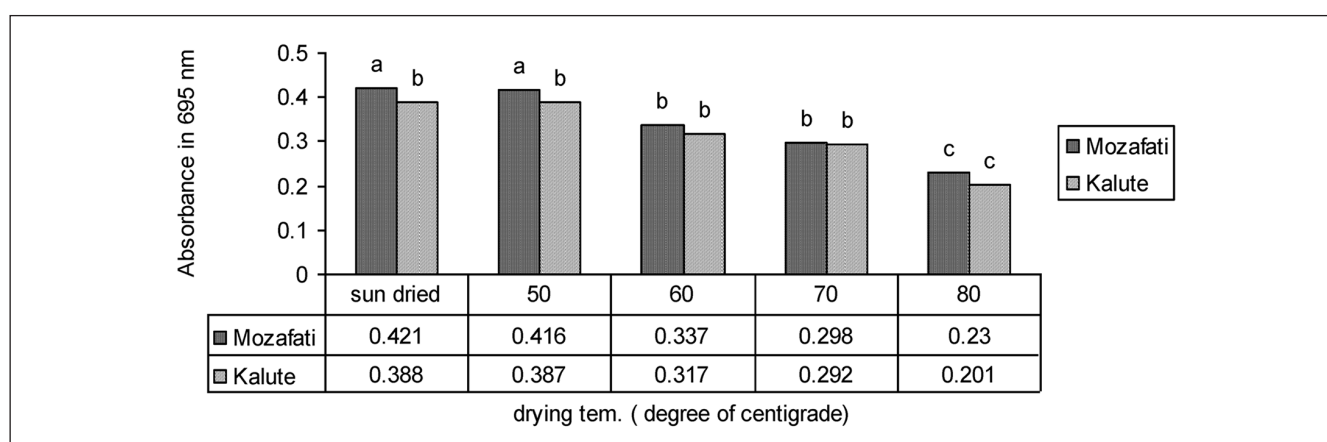


Figure 4.—Effect of drying process on the total antioxidant capacity of dried date (500 ppm concentration).

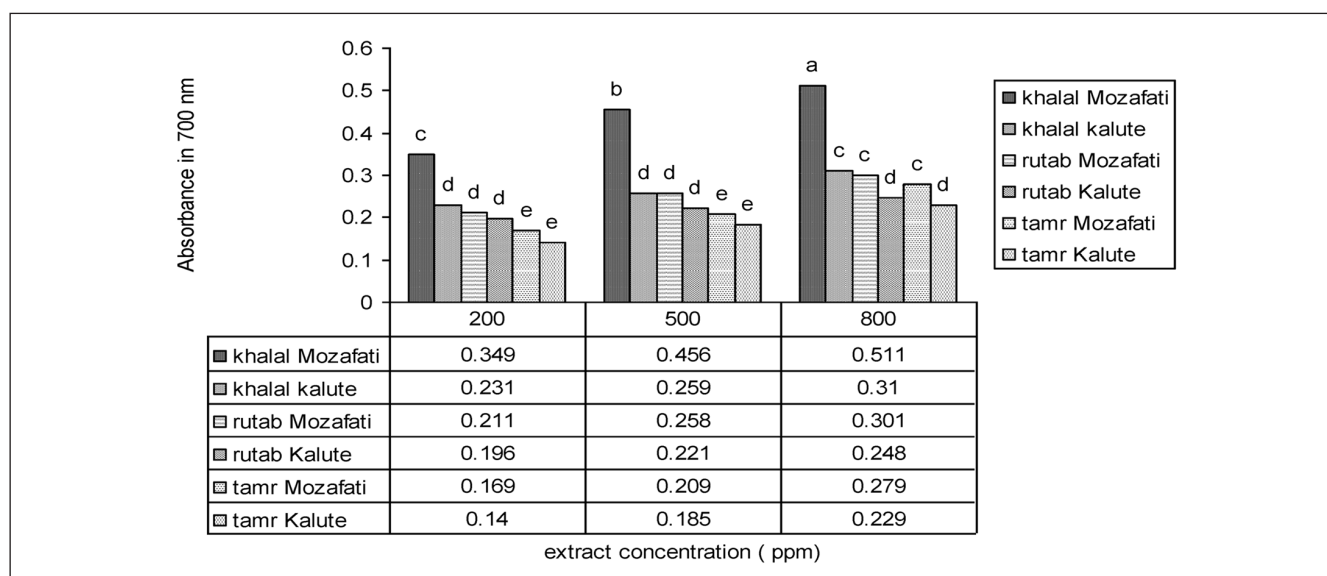


Figure 5.—Effect of ripening stages on reducing power of date extracts.

In this study extracts reducing power were showed significant differences ($P < 0.05$) from khalal stage to rutab stage but no significant differences ($P < 0.05$) in rutab stage and tamr stage were observed. Khalal stage containing the highest amount of total phenolics, was the most potent reducing agent, whereas tamr stage containing the least amount of phenolics, was the weakest in the reducing activity. Similar relations between iron (III) reducing activity and total phenol content have been reported in the literature;^{39, 40} however the correlation may not be always linear.²⁸

The results of the effect of drying temperature on reducing power of dried dates in concentration of 500 ppm were shown in Figure 6.

As can be seen from Figure 6, reducing power of date extracts decreased by increase of drying temperature and no significant differences ($P < 0.05$) observed between sun dried date and oven (50 °C) dried date in case of reducing power. Mozafati had higher reducing power than kalute ($P < 0.05$). Arab-shahi-Delouee and Urooj (2009) reported similar results about the effect of temperature on antioxidant reduction of mulberry (*Morus indica* L.) leaves.²⁶

DPPH RADICAL SCAVENGING ACTIVITY

The results of DPPH radical scavenging activity of extracts affected by ripening stages were shown in Figure 7. Free radicals which are involved in the process of lipid per-oxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others

(441). The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids.⁴²

As it can be seen from Figure 7, Khalal stage that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by rutab stage and tamr stage. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits.^{26, 40} Figure 7 also showed that mozafati date had higher DPPH (%) than kalute variety in all ripening stage ($P < 0.05$). The results of DPPH radical scavenging activity of dried date extracts (500 ppm) were shown in Figure 8.

This figure showed that DPPH radical scavenging decreased by increasing drying temperature and no significant differences ($P < 0.05$) were found between DPPH radical scavenging of sun dried date and dried date with oven (50 °C). These results may be due to reduction of phenolic compound by temperature.²⁶

Conclusions

The antioxidant activities (AA) and total phenolic content (TPC) of Kerman dates were determined and presented in this paper. Two dates variety had significant difference in case of antioxidant activity and total phenolic content ($P < 0.05$) and mozafati variety had higher that kalute in these case. In both varieties antioxidant activities (AA) and total phenolic content (TPC) decreased by ripening stages

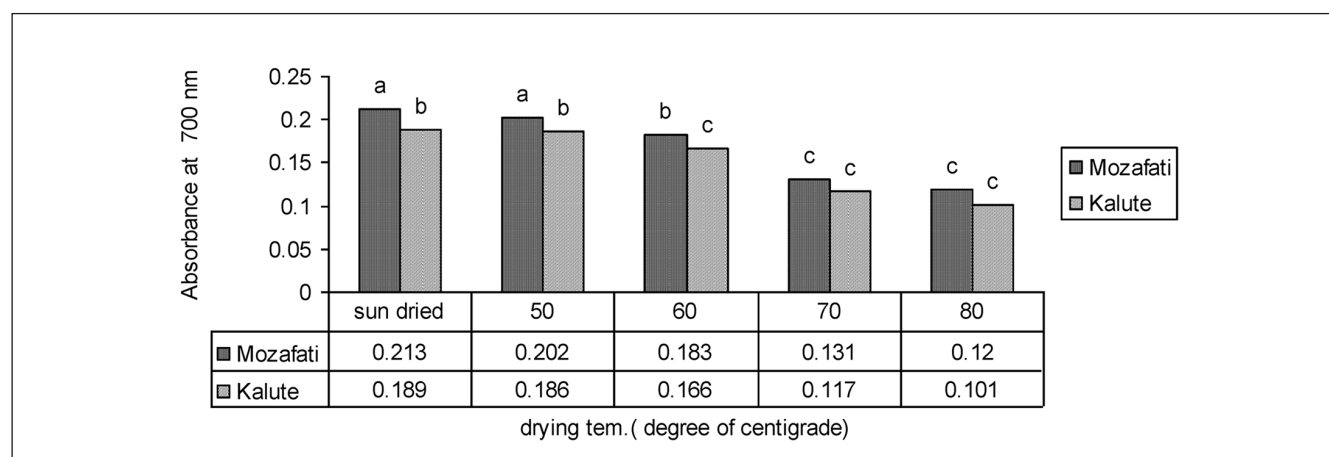


Figure 6.—Effect of drying temperature on reducing power of dried date extract (500 ppm concentration).

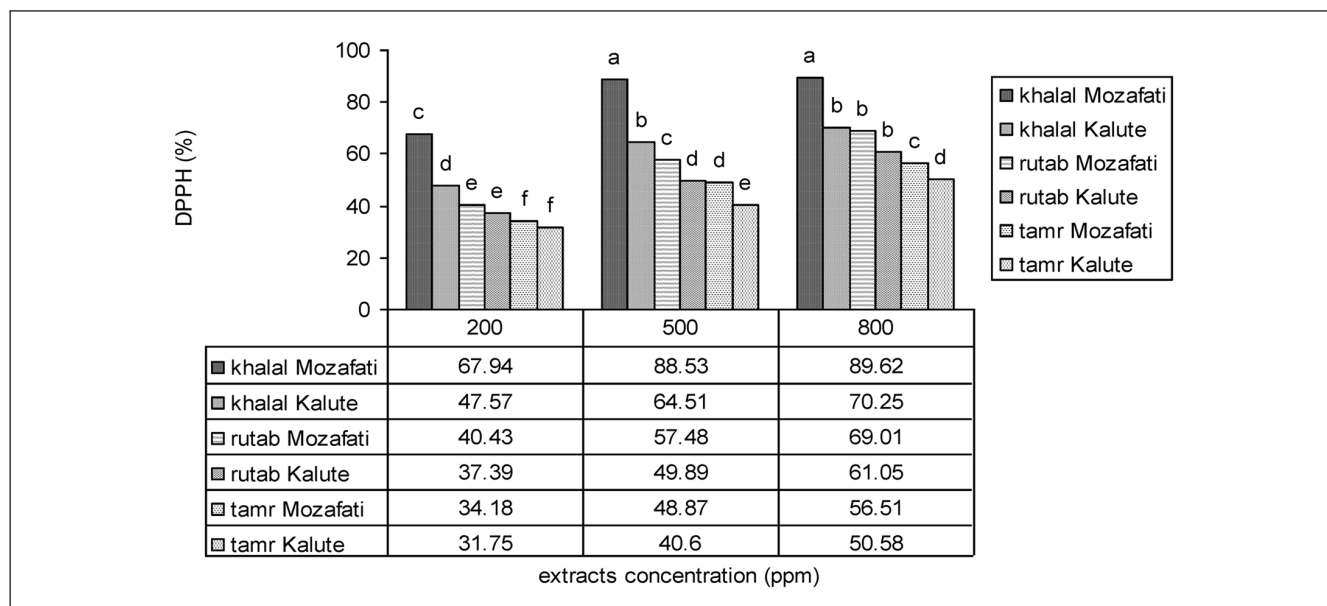


Figure 7.—DPPH radical scavenging activity of date extracts in ripening stages.

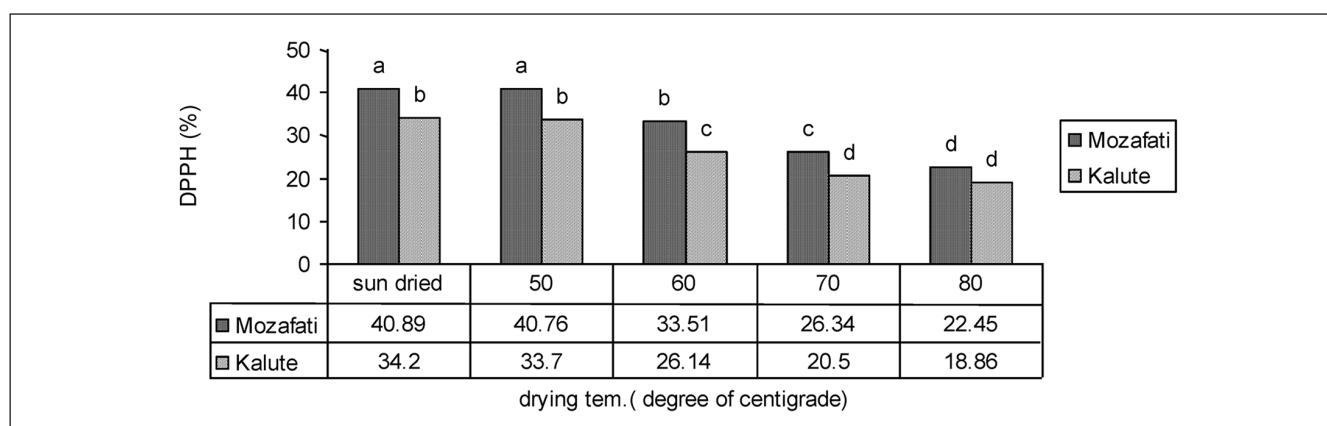


Figure 8.—DPPH radical scavenging activity of dried date extracts (500 ppm).

and Khalal stage contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. Result of drying process showed that total phenolic content (TPC) and antioxidant activities (AA) varied with temperature and decreased by increase of drying temperature.

This fruit could be potential rich resources of natural antioxidants, and could be developed into functional foods or drug for the prevention and treatment of diseases caused by oxidative stress. In the

future, the specific components with high antioxidant capacities in fruits should be isolated and identified, and explored for their health effects against oxidative stress.

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